McMASTER

LOSS OF URANIUM BIOADSORPTIVE CAPACITY ON RECYCLE

FINAL REPORT

by

MARIOS TSEZOS

CHEMICAL ENGINEERING

McMASTER UNIVERSITY

HAMILTON, ONTARIO

CANADA

OCTOBER 1989

Engineering

CONTRACT SERIAL NO. 89082-01-SS
REQUISITION NUMBER 23440-8-9082
CONTRACTOR: MCMASTER UNIVERSITY

LOSS OF URANIUM BIOADSORPTIVE CAPACITY ON RECYCLE

FINAL REPORT

by

MARIOS TSEZOS

CHEMICAL ENGINEERING
McMASTER UNIVERSITY
HAMILTON, ONTARIO
CANADA

SSC Contract File: 063SS.23440-8-9082

Scientific Authority: Dr. R.G.L. McCready, Head CANMET Biotechnology Section

OCTOBER 1989

40 pp

MICROMEDIA

90-02541/

EXECUTIVE SUMMARY

Previous studies by Dr. M. Tsezos (DDS contract file 388 ST. 23440-6-9164) have shown that a reduction in the uranium uptake capacity occured after the immobilized R. arrhizus biomass was subjected to repeated loading and elution cycles using Denison Mines uranium bioleach solutions. In order to determine the possible causes of uptake reduction, the immobilized biomass from (both batch and continuous column) loading-elution cycling experiments was studied using transmission electron microscopy (TEM), x-ray energy dispersion analysis (EDAX) and infrared spectroscopy (IR). The results indicate that the reduction in uptake may be due to the bioleach solution composition, since the decrease in uptake was far less pronounced than previously observed with a different solution sample. TEM and EDAX showed that aluminum was present in the biomass cell wall and was not eluted. The IR study was not conclusive at the available level of resolution.

RÉSUMÉ EXÉCUTIF

Les études antérieures par Dr. M. Tsezos (CONTRAT DSS FICHIER 388 ST. 23440-6-9164) ont démontrées qu'une réduction dans la capacité d'apport de l'uranium a lieu après que la biomasse immobilisée R. arrhizus a été sujet à des cycles répétés de chargement et élution avec une solution d'uranium lessivée de façon biologique provenant de Denison Mines. Afin de déterminer les raisons possibles pour la réduction de l'apport, la biomasse immobilisée, provenant des expériences (à colonne discontinue et continue) de fluctuation en chargement-élution, a été étudiée à l'aide de la microscopie électronique de transmission (TEM), l'analyse de dispersion d'énergie des rayons X (EDAX) et la microscopie infrarouge (IR). Les résultats indiquent que la réduction en apport peut-être dû à la composition de la solution lessivée de façon biologique, puisque la décroissance en apport était beaucoup moins prononcée que ce qui était observée antérieurement avec un autre échantillon de solution. TEM et EDAX ont demontrées que l'aluminium et présent dans le mur cellulaire de la biomasse et n'a pas été élué. L'étude IR n'était pas conclusive au niveau de resolution disponible.

TABLE OF CONTENTS

1. Introduction 1

    1.1 Project Scope and Objectives 1

2. Materials and Methods 2

    2.1 Biomass Characteristics 2

    2.2 Biosorption Isotherms 3

3. Results and Discussion 7

    3.1 Batch Loading and Elution Cycling 7

    3.2 Continuous Loading and Elution Cycling 8

    3.3 Biomass Observations and Analysis 9

        3.3.1 Transmission Electron Microscopy and Microprobe Analysis 9

        3.3.2 Infrared Spectroscopy 20

4. Conclusions 34

References 35

LOSS OF URANIUM BIOADSORPTIVE CAPACITY ON RECYCLE

1. INTRODUCTION

1.1 Project Scope and Objectives

Previous experimental work (DSS contract file 388 ST.23440-6-9164) suggested that an initial decrease in uranium uptake capacity occurred when immobilized R. arrhizus biomass was subjected to repeated uranium loading and elution cycles using Dension Mines biological leachate. Extensive experience in this area and the detailed mechanistic work underway in the area of uranium biosoption by Dr. M. Tsezos, resulted in the Department of Supply and Services Canada and Dr. M. Tsezos of McMaster University signing contract file 23440-8-9082/01-SS entitiled "Loss of Uranium Bioadsorptive Capacity on Recycle". This project has as its objective to initiate work in the direction of the mechanistic understanding of the factors that result in the above mentioned biosorptive capacity reduction. The specific objectives of the present work are as follows:

1) To produce, process and immobilize R. arrhizus biomass using the proprietary technology developed by Dr. M. Tsezos.

2) Using industrial bioleach solution supplied by Denison Mines from the Elliot Lake area, to load the immoblilized R. arrhizus biomass particles with uranium.

3) Examine samples of the loaded immobilized biomass using Electron Microscopy and Infrared Spectroscopy.

4) Elute the same immobilized biomass particles with sodium bicarbonate and examine the eluted biomass as in objective (3).

5) Repeat the above to a total of three cycles and evaluate the results.

In the course of the present work the immobilized R. arrhizus biomass was subjected to three cycles of loading and elution in batch mode and, in addition, to several continuous column uranium loading/elution cycles. Immobilized biomass samples from different points in the cycling were analyzed using transmission electron microscopy, x-ray energy dispersion and infrared spectroscopy. Furthermore, the immobilized biomass uranium equilibrium uptake isotherms were considered necessary and were experimentally determined using Dension Mines bioleach solution adjusted to pH 4.0.

2. MATERIALS AND METHODS

2.1 Biomass Characteristics

The biomass used in these experiments was R. arrhizus, a fungus. It was produced, processed and immobilized using a proprietary process developed at McMaster University by Dr. M. Tsezos. The biomass pellets were size classified to produce particles of 20 - 25 mesh size. Approximately 12% of the particles' final weight consists of polymeric material additives. The biomass produced in an

earlier CANMET contract (DSS contract file 23440-8-9069/01-SS) also had the 12% polymer content, but was size classified to produce particles of size 18 - 30 mesh.

2.2 Biosorption Isotherms

Uranium isotherms were developed by contacting the immobilized biomass with a given volume of the Denison Mines process solution after adjusting the pH to 4.0 and filtering the resulting precipitate. Weighed amounts of immobilized biomass were placed into screwtop flasks with 100 mL of the processed bioleach solution. The flasks were then shaken for 21 days on an orbital shaker. At the end of the contact time the solutions were then filtered, using 0.45 μm Millipore filter paper. The filtrates were analyzed for uranium by delayed neutron counting. The immobilized biomass uranium uptake capacity was subsequently determined by a uranium mass balance.

Two different samples of immobilized biomass were examined and the results of the respective uptake capacity tests are tabulated in Table 1. Both sets of data were determined using the same samples of processed bioleach solution at pH 4.0. Figure 1 presents the corresponding adsorption isotherms.

The uptake capacity of the two batches is very similar. Both isotherms show relatively high uptake at low solution concentrations and then exhibit a relatively robust uptake of about 140 mg/g. These isotherms are close to the isotherms determined for the batch of biomass produced for the previously mentioned work (DSS contract file 23440-8-9069/01-SS), thus confirming the

consistency of the uranium biosorptive properties of the produced R. arrhizus immobilized biomass particles.

TABLE 1

MINE SOLUTION ISOTHERM DATA

BATCH 1 BIOMASS
MINE SOLUTION: 284 PPM INITIAL U CONCENTRATION

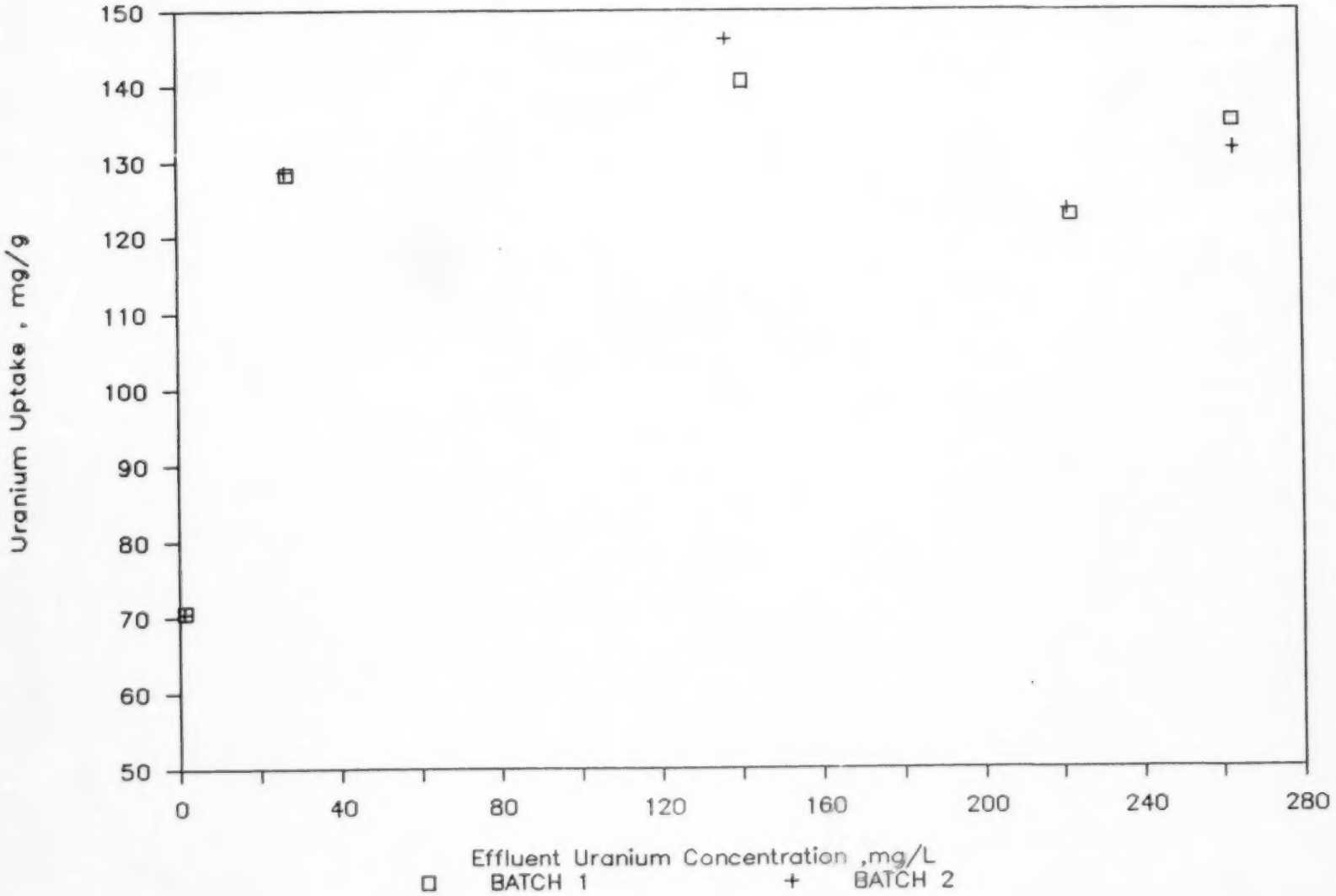
Residual bulk U concentration	U Uptake mg/g
1.31	70.6
27.18	128.3
140.56	140.6
222.13	122.8
243.16	135.2

BATCH 2 BIOMASS
MINE SOLUTION: 284 PPM INITIAL U CONCENTRATION

Residual bulk U concentration	U Uptake mg/g
1.28	70.6
26.44	128.7
136.43	146.1
221.45	123.6
244.24	131.6

The bioleach solution was processed by adjusting the pH to 4.0 through a drop wise addition of sodium hydroxide and mixing thoroughly. The resulting precipitate was allowed to settle before filtering the clear supernatent solution through a glass fibre filter and a 0.45 μm Millipore filter. An analysis of the processed bioleach solution is given in Table 2. Delayed neutron counting was used to determine uranium solution concentrations while the Aresnazo III

Figure 1: Uranium Biosorption Isotherms



colorimetric technique was used as a reference. DCP analysis was used for the analytical determination of the remaining elements.

TABLE 2

ANALYSIS OF MINE WATER AFTER pH ADJUSTMENT

Element	Feed ppm
U	287.000
Al	16.500
Ca	155.000
Cd	0.070
Cr	0.100
Cu	1.060
Fe	0.755
Mn	2.600
Ni	2.450
Pb	0.350
Zn	16.200
Co	3.400
Ti	< 0.100
Sb	< 0.250
Mo	< 0.500
B	1.500
Au	< 0.500
Ag	< 0.100
Si	24.200
Be	< 0.050
Ti	< 0.050
Ba	< 0.050
Li	< 0.100
Se	0.007
Sn	< 0.100
K	14.500
Mg	26.600
Na	2678.000
pH	4.0

3. RESULTS AND DISCUSSION

3.1 Batch Loading and Elution Cycling

Three cycles of batch loading and elution were performed in duplicate on the immobilized biomass. Each time, a weighed amount of biomass was placed in a contact vessel with 100 mL of processed bioleach solution, and contacted for seven days using an orbital shaker. The biomass was then separated from the solution by filtering with a 0.45 µm Millipore filter. The biomass was washed with 250 mL of distilled water and returned to the contact vessel to which was added 120 mL of 0.5 N sodium bicarbonate solution. The vessel was then shaken for three days to elute the sorbed uranium. The biomass was then separated again from solution by filtration and washed with distilled water. This loading and elution process was repeated for three cycles. All loading and elution filtrates as well as was wash solutions were retained for analysis. Three cycles were chosen since the drop in uptake previously observed (DSS contract file 23440-6-9164) was observed in the first three cycles.

The results from the previous work show that unlike the earlier tests, a significant drop in the immobilized biomass uranium biosorptive uptake capacity did not occur during the first three cycles, although the corresponding initial equilibrium uptake should have been about 140 mg/g as suggested by the biosorption isotherm. At the same time, the quantity of uranium eluted in each cycle is consistent with that obtained in earlier tests, indicating that, as before, uranium is recovered completely. After completion of these tests the biomass was air dried

and weighed. On the average, approximately 16% of the original biomass weight was lost during the test. The results of the batch tests are summarized in Table 3.

TABLE 3

BATCH LOADING AND ELUTION CYCLING RESULTS

TEST 1: Initial Sorbent Mass 201.0 mg
Final Sorbent Mass 175.1 mg

Run	Uranium Uptake mg/g	Uranium Eluted mg/g	Uranium Eluted %
1	125	120	96
2	127	128	101
3	125	124	99

TEST 2: Initial Sorbent Mass 200.0 mg
Final Sorbent Mass 160.4 mg

Run	Uranium Uptake mg/g	Uranium Eluted mg/g	Uranium Eluted %
1	126	115	91
2	124	122	99
3	127	126	100

3.2 Continuous Loading and Elution Cycling

In addition to the batch tests, six cycles of continous loading and elution were performed using the same Denison Mines bioleach solution adjusted to pH 4.0. The immobilized biomass uranium uptake of the fifth and sixth cycles was 125 mg/g and 120 mg/g respectively. These immobilized biomass uranium loadings are

similar to those observed during the first three batch uranium loading/elution tests. Comparison of the data to the corresponding biosorption isotherm suggests that a decrease in the equilibrium uptake has occured, but it is less pronounced than previously observed (DSS contract file 23400-6-9164). This may be due to differences in the composition of the mine water as the sample used for the present tests was obtained at a later time and it was different than the one used for the previous work (DSS contract file 23440-6-9164). Significant variations in the leach liquor mine water composition are known to occur not only as a function of the time of the year (seasonal changes) but also as a function of the location of the stope in the production in the mine.

3.3 Biomass Analysis

3.3.1 Transmission Electron Microscopy/Microprobe Analysis

The biomass used in the continuous loading-elution cycles was examined using electron microscopy and x-ray energy dispersion analysis. The biomass samples were dehydrated with a series of ethyl alcohols and embedded in Spurr epoxy resin. An LKB Microtome III was used to cut sections approximately 800 Å thick. These sections were then mounted on copper grids. X-ray energy dispersion spectra were recorded using the TEM sections. The microprobe was focused at a magnification of 100,000X at an accelerating voltage of 80 kV.

Electron micrographs, in Figures 2,3 and 4 enable comparison between the typical R. arrhizus cell wall appearance before uranium biosorption, after

uranium loading and after uranium elution, respectively. Figure 2 shows, as expected, that there are no electron dense layers in the virgin biomass cell wall. Figure 3, a typical electron micrograph of the fungal cell wall following uranium loading, shows the expected stratified dark electron dense layers produced by biosorbed uranium inside the cell wall. The stratified layers are similar to those observed in earlier work (Tsezos and Volesky, 1982). Microprobe anaylsis of the virgin biomass shows no uranium or aluminum inside the cell wall (Figure 5). Figure 4 illustrates typical electron micrographs of R. arrhizus cell walls after elution at the end of the third cycle and suggest that unlike the previous experience where pure uranyl nitrate solutions have been used, electron dense stratified layers remained inside the cell wall after elution, although all the biosorbed uranium had been eluted as shown by the mass balance on Table 3. Microprobe anaylsis of the dark areas indicated the presence of both aluminum and uranium after loading (Figure 6). However, microprobe anaylsis after elution (Figure 7) confirmed that all the uranium had been removed during elution, but that aluminum remained present inside the cell wall.

Figure 2

Virgin R. arrhizus mycellium section before uranium biosorption. (10.5X)

Figure 3

R. arrhizus cell wall following uranium biosorption from Denison Mines biological leachate at pH 4.0. (50K)

Figure 4

R. arrhizus cell wall following elution of uranium previously biosorbed from Dension Mines biological leachate. (48K)

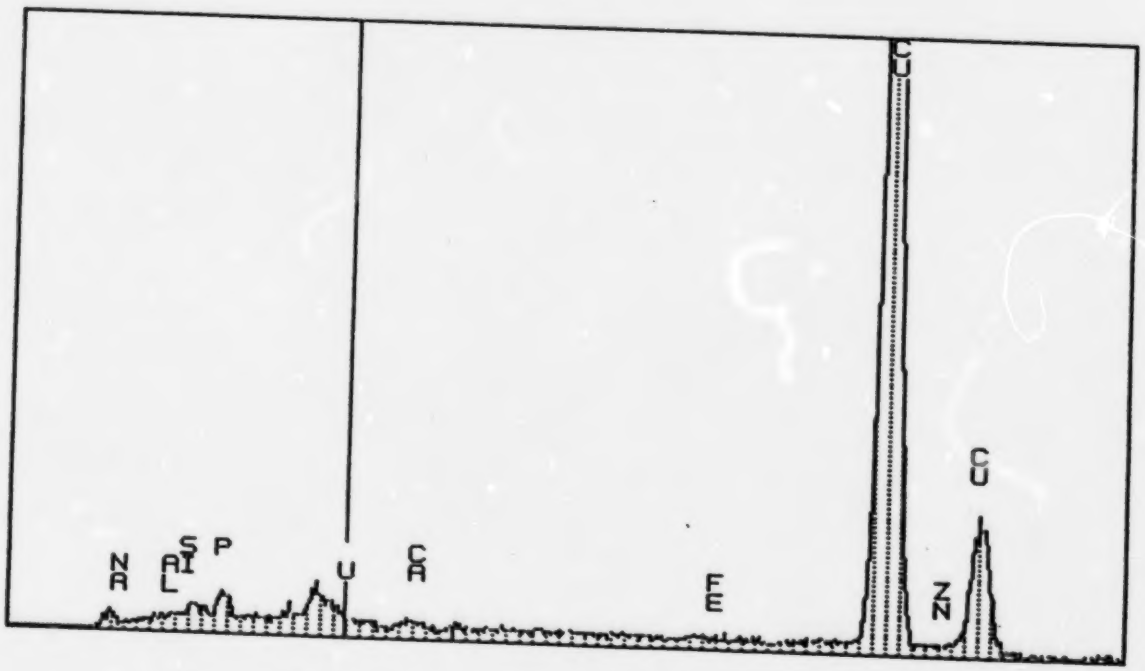


Figure 5

X-ray Energy Dispersion Analysis Spectrum of R. arrhizus virgin immobilized biomass.

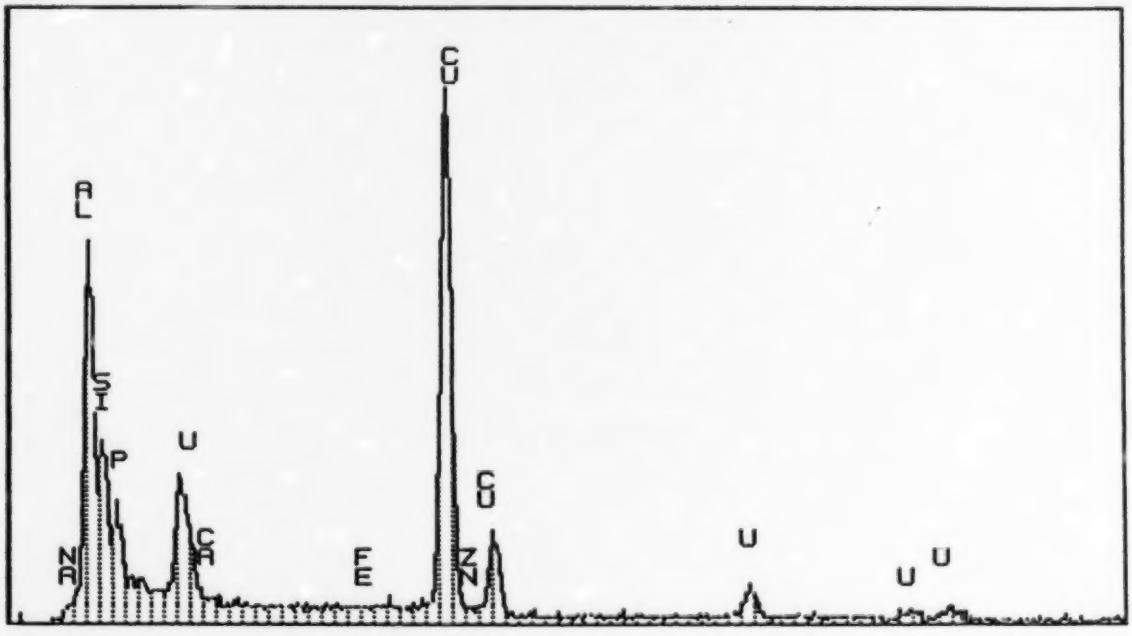


Figure 6

X-ray Energy Dispersion Analysis Spectrum of R. arrhizus immobilized biomass following uranium uptake from Denison Mines biological leachate.

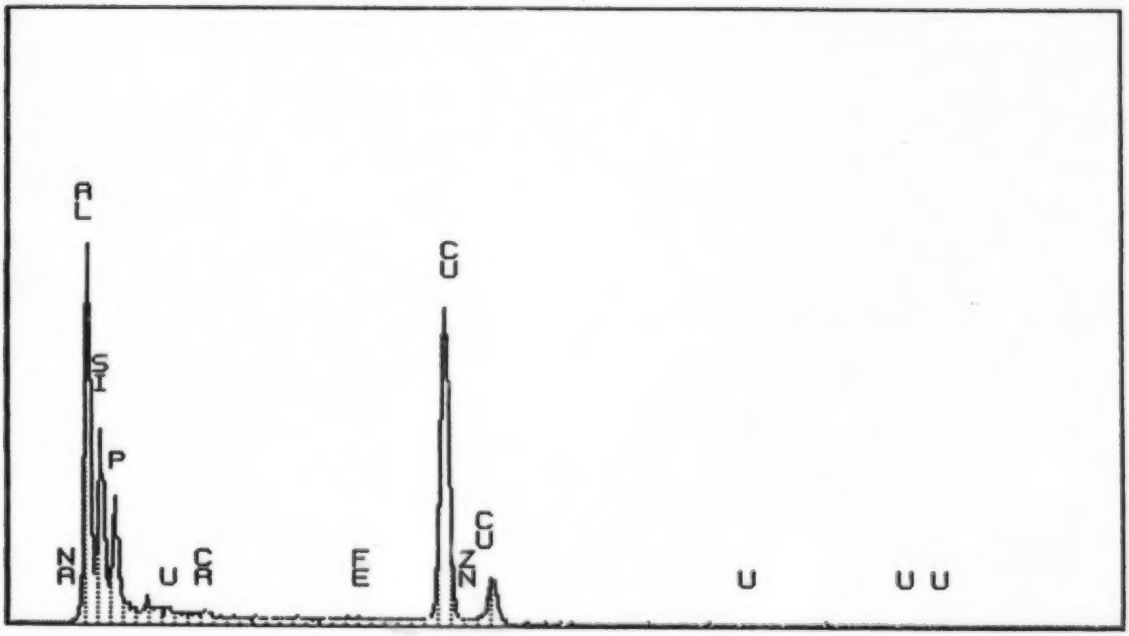


Figure 7

X-ray Energy Dispersion Analysis Spectrum of R. arrhizus immobilized biomass after uranium elution. Denison Mines biological leachate.

3.3.2 Infrared Spectroscopy

Infrared spectroscopy was used in an effort to determine the nature of possible chemical interaction between aluminum and the cell wall. The spectra were recorded with a Perkin-Elmer 297 infrared spectrometer. All spectra were obtained using KBr discs. Spectra of virgin R. arrhizus both immobilized and non-immobilized were also determined and are presented along with spectra of the biomass after several cyclical contacts with bioleach solution. The spectra are presented individually in Figures 8-12, while Figure 13 presents all the above spectra aligned together in order to facilitate the comparison.

There appears to be little difference among the spectra of immobilized and non-immobilized virgin biomass samples. This means that, as expected, the immobilization process had no discernable effect on the structure of the immobilized mycellia. This is an additional confirmation of the advantage this implemented immobilization process has in that it maintains intact the structural characteristics of the biomass that is being immobilized. This is an important requirement for optimizing the biosorptive behaviour of any immobilized biomass particles. A comparison of the virgin R. arrhizus IR spectrum to that of the biomass following the first contact with the bioleach solution (first uranium loading) shows only one obvious difference, the expected characteristic v_3 uranyl ion peak at 908 cm^{-1}. Comparison of the IR spectra of the virgin immobilized biomass (MSR) to that of the immobilized biomass following several bioleach solution biosorption cycles (Cont L16) reveals a much broader peak in the 900-1200 cm^{-1} region while at the same time the uranium v_3 peak is not evident. It is not clear whether the above

mentioned broad peak masks the v_3 uranium peak or whether the v_3 uranium peak is absent or weakened. The above analysis of the spectra suggests that a much higher resolution spectrophotometer (FTIR spectrometer) should be used in order to attempt to derive more specific conclusions.

Figure 8

Infrared spectrum of virgin R. arrhizus mycellia extracted from virgin immobilized R. arrhizus particles. (Native Ra.)

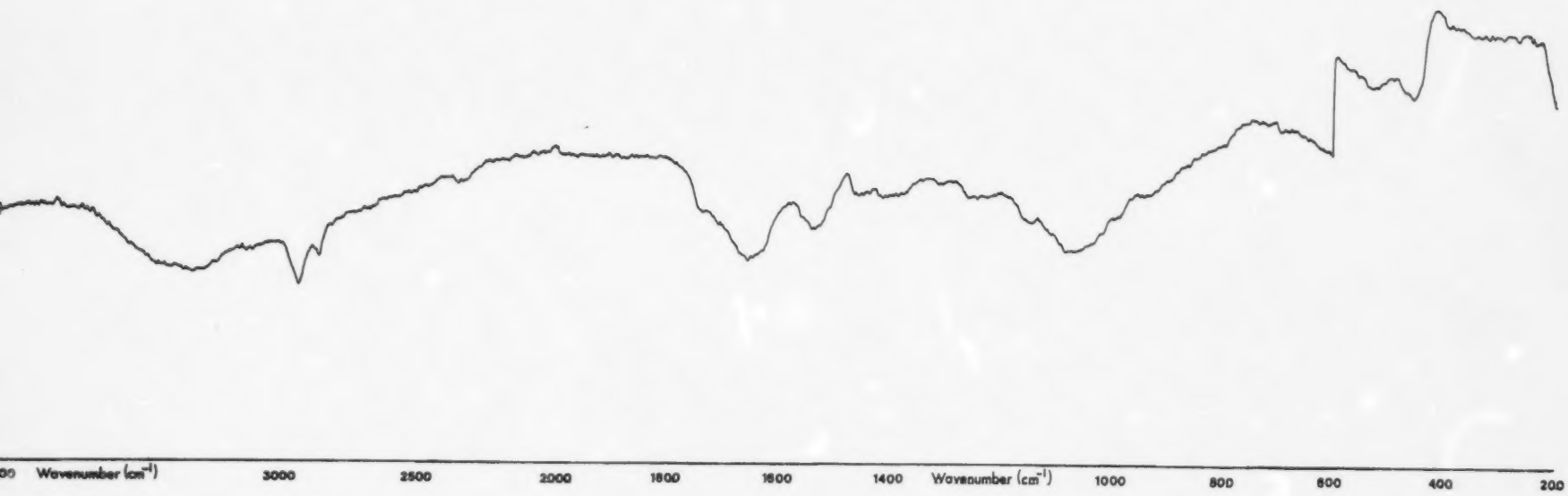

00
Wavenumber (cm⁻¹)
3000
2500
2000
1800
1600
1400
Wavenumber (cm⁻¹)
1000
800
600
400
200

Figure 9

Infrared spectrum of virgin immobilized R. arrhizus particles. (MSR)

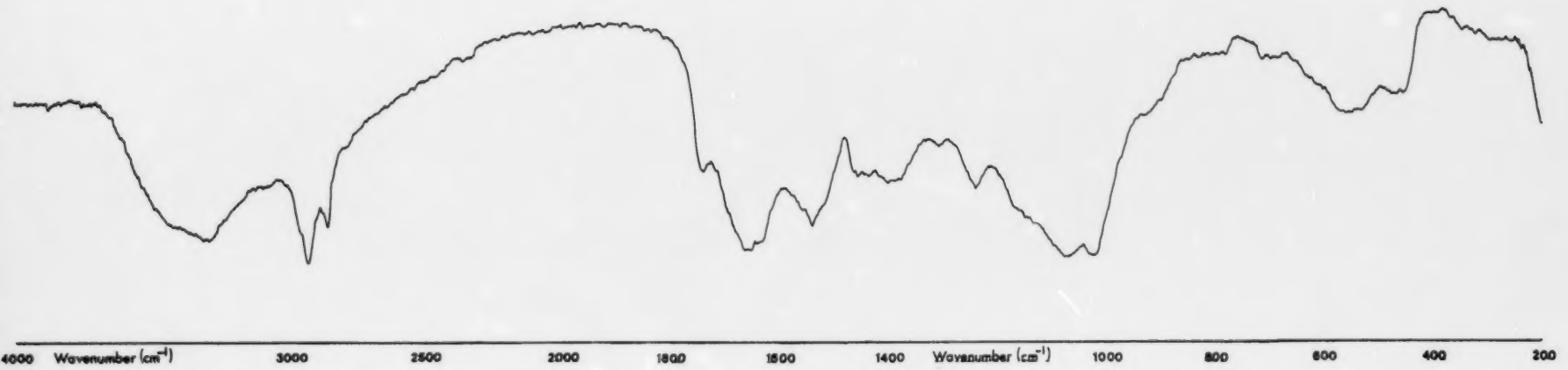

4000
Wavenumber (cm⁻¹)
3000
2500
2000
1800
1500
1400
Wavenumber (cm⁻¹)
1000
800
600
400
200

Figure 10

Infrared spectrum of R. arrhizus immobilized biomass particles following the first loading with uranium from Denison Mines biological leachate at pH 4.0. (Batch L1)

4000
Wavenumber (cm⁻¹)
3000
2500
2000
1800
1600
1400
Wavenumber (cm⁻¹)
1000
800
600
400
200

Figure 11

Infrared spectrum of R. arrhizus mycellia extracted from MSR particles following uranium loading after several continuous uranium biosorption/elution cycles. (Cont. L16 interior)

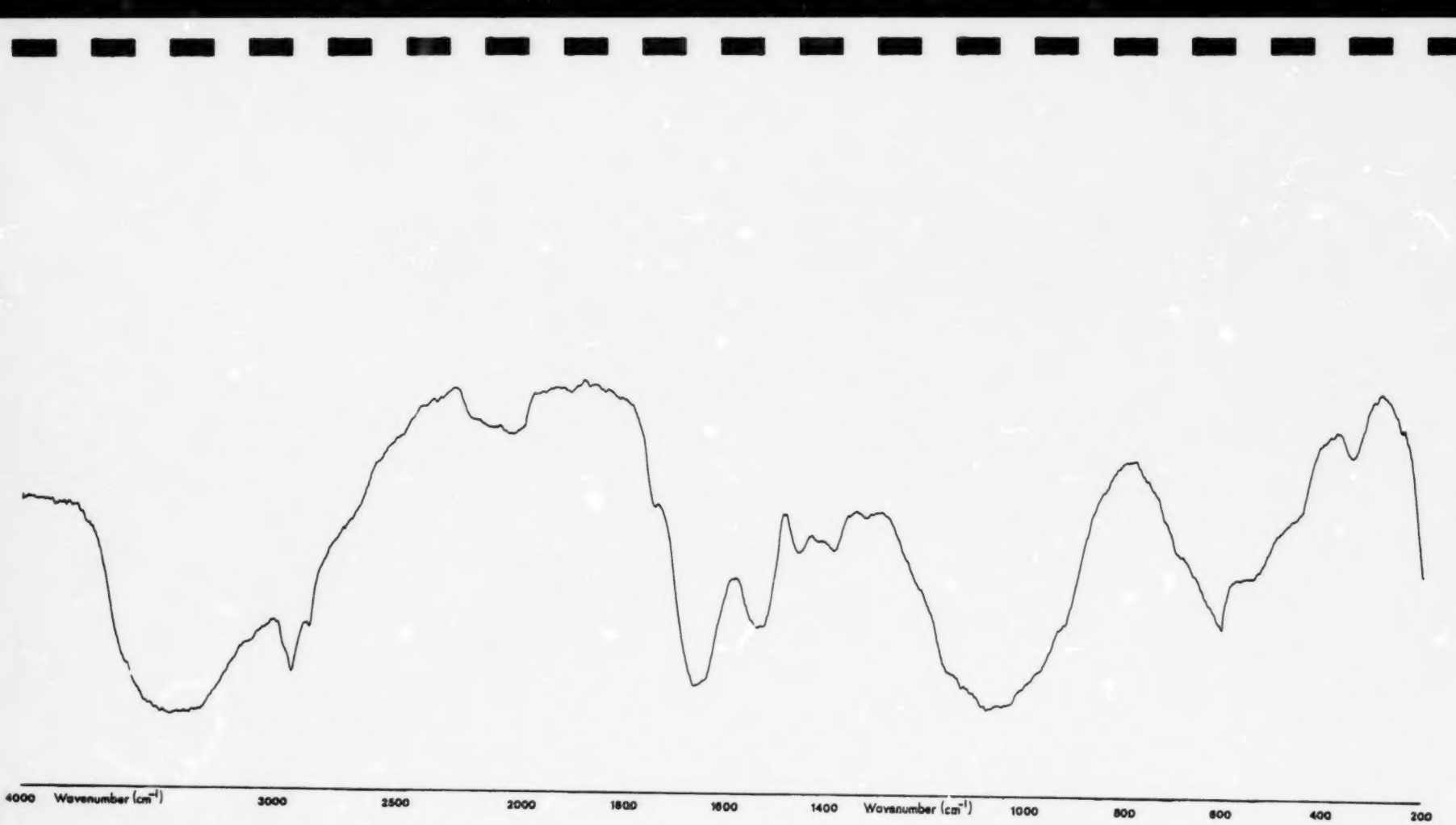

4000
Wavenumber (cm⁻¹)
3000
2500
2000
1800
1600
1400
Wavenumber (cm⁻¹)
1000
800
600
400
200

Figure 12

Infrared spectrum of R. arrhizus immobilized biomass particles (MSR) after uranium loading and following several continuous uranium biosorption/elution cycles (Cont. L16)

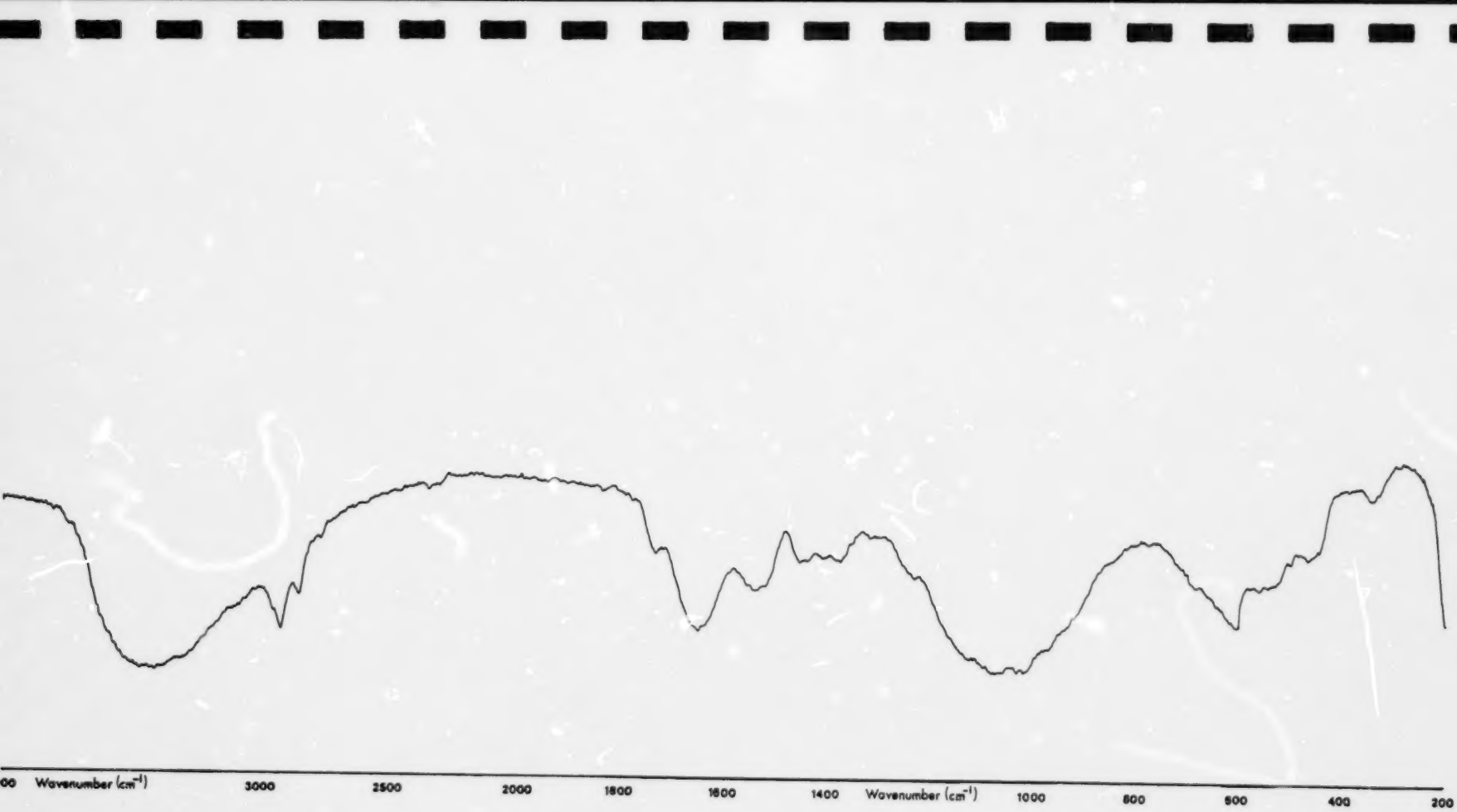

00
Wavenumber (cm⁻¹)
3000
2500
2000
1800
1600
1400
Wavenumber (cm⁻¹)
1000
800
600
400
200

Figure 13

Comparison of Infrared spectra of:

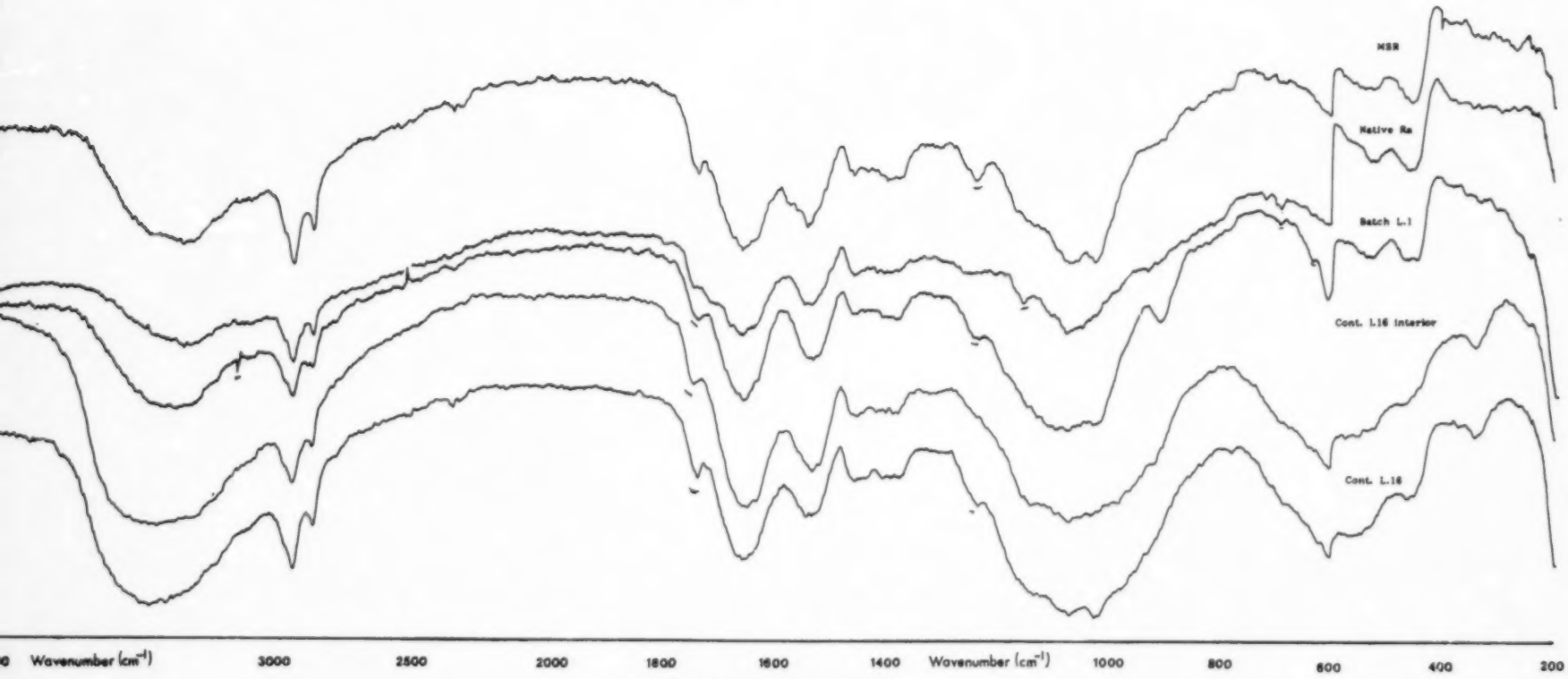
MSR: virgin immobilized biomass particles

Native Ra: virgin R. arrhizus mycellia extracted from virgin MSR particles

Batch L1: MSR particles following the first uranium loading from Denison Mines bioleachate

Cont. L16 interior: R. arrhizus mycellia extracted from MSR particles following uranium loading after several continuous uranium biosorption/elution cycles

Cont. L16: MSR particles following uranium loading after several continuous biosorption/elution cycles



IR SPECTRA U/Al COMPETITIVE BIOSORPTION

7. CONCLUSIONS

The results from the present experimental work suggest that:

(1) A decrease in the uranium uptake of the immobilized biomass was observed. However, it was far less pronounced than that observed previously. This could be attributed to differences in the mine water composition.

(2) Aluminum was biosorbed from the bioleach solution by the immobilized biomass and was not released during elution.

(3) The IR spectroscopy study was not conclusive at the available level of resolution. Higher resolution IR spectroscopy studies will be required in order to obtain better information.

REFERENCES

1. Tsezos, M. "The Use of Immobilized Biomass to Recover Uranium from Elliot Lake Type Ores", DSS Contract File 388 ST. 23440-6-9164, April 1988.

2. Tsezos, M. "Preparation of 1.3 kg of Immobilized R. Arrhizus for Biomass Adsorption", DSS Contract File 23440-8-9069/01-SS, January 1989.

3. Tsezos, M. and Volesky, B. "The Mechanism of Uranium Biosorption", Biotechnology and Bioengineering, 24, pp. 385-401, 1982.